

THE EFFECT OF MUSCIMOL, A GABA AGONIST, ON THE ACQUISITION OF
LEARNING SET IN MALE LONG-EVANS RATS

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Abstract

Fourteen male Long-Evans rats were randomly assigned to one of two groups: saline (n = 7) or muscimol (n = 7). The rats received 0.5 mg/kg of muscimol (intraperitoneal, i.p.) or saline (i.p.) during the initial 40 odor-unique discrimination problems to test effects of muscimol on the acquisition of learning set. Muscimol did not impair the acquisition of a learning set, as evidenced by the significantly higher than expected by chance Trial 2 performance. Muscimol also did not result in performance or motor deficits, or changes in emotionality.

The Effect of Muscimol, a GABA Agonist, on the Acquisition of Learning Set in Male Long-Evans Rats

Alzheimer's disease (AD), though intensely studied, still plagues today's aging population. AD is a progressive degenerative disorder of the brain, and is characterized by a marked deterioration of cholinergic neurons in the brain, mainly in the hippocampus, cortex, and the basal forebrain (Bear, Connors, & Paradiso, 2001; Morón et al., 2002). Because the cholinergic cells deteriorate in the brain of a patient with AD, past research has focused mainly on the role of acetylcholine in the progression of AD.

Past research has shown that the decreased levels of acetylcholine in the basal forebrain are responsible, at least in part, for the cognitive deterioration seen in AD (Robner, Schliebs, & Bigl, 1994). In rats, research has focused mainly on the nucleus basalis magnocellularis (nBM), located in the caudal region of the basal forebrain, because it directly parallels the human nucleus basalis of meynert (Fibiger, 1982; Manfredi, Brambilla, & Mancina, 2001). Lesions in the nBM produce deficits in various learning paradigms, such as learning set formation (Bailey & Thomas, 2001) and radial arm maze performance (Wellman & Pelleymounter, 1999). Furthermore, damage in the medial septal area, also part of the basal forebrain cholinergic system, produces learning deficits similar to lesions in the nBM (Wenk, Hughey, Boundy, & Kim, 1987).

Nonspecific neurotoxic lesions to the nBM have been shown to significantly impair learning set formation (Bailey & Thomas, 2001). Learning set has been defined as "learning how to learn efficiently in a situation an animal frequently encounters" (Harlow, 1949, p. 51) or as a win-stay/lose-shift hypothesis (Levine, 1965). This hypothesis predicts that if an animal is presented with two objects and it responds to the correct object on Trial 1 and is rewarded, it

should stay with this choice on subsequent trials. However, if the animal chooses incorrectly on Trial 1, it should learn to shift its response to the other choice on subsequent trials. Therefore, it can be said that the animal has successfully formed a learning set if it performs significantly above chance (50%) on Trial 2 on a series of discrimination problems.

Bailey and Thomas (2001) concluded that the nBM is critical for learning set formation due to the animals' inability to acquire a learning set following non-selective lesions to the nBM. However, Bailey, Rudisill, Hoof, and Loving (2003) found that 192-IgG saporin cholinergic lesions resulted in deficits, lowering learning set performance, but did not block acquisition. Thus, despite the loss of a large number of cholinergic neurons located in the nBM, animals could still form and use a learning set. These results suggested that another neurochemical system may be critical for the acquisition and use of learning set.

In addition to the large body of cholinergic neurons in the nBM, there are also a large number of GABAergic neurons located within the nBM. These GABAergic neurons in the nBM outnumber the cholinergic neurons 2:1 (Gritti, Mainville, & Jones, 1993). Research has shown that GABAergic neurons from the nucleus accumbens synapse on cholinergic cells in the nBM, which may have some effect on the reinforcement of learning tasks (Zaborsky, Cullinan, & Braun, 1991; as cited in Mayo et al., 1993). Additionally, Martin, Lee, Grabill, and Bailey (2003) found improvement in learning set performance in nBM-lesioned animals following injections of flumazenil, a GABA antagonist, again suggesting a role for the GABAergic system in learning set performance.

Numerous studies have shown deficits in the acquisition of various learning paradigms following muscimol injections, such as taste and place aversive learning (Morón et al., 2002), radial arm maze (Majchrzak, Brailowsky, & Will, 1990), passive avoidance (Majchrzak et al.,

1990), and fear conditioning (Muller, Corodimas, Fridel, & LeDoux, 1997). Injections of 1.0 and 2.0 mg/kg of muscimol, a GABA agonist, have also previously been shown to impair aspects of learning and memory including the retention of inhibitory avoidance (Castellano & McGaugh, 1990; Morón et al., 2002), retention of latent inhibition (Holt & Maren, 1999), retention of reward memory (Salinas & McGaugh, 1995), and overall place learning in the water maze (Nakagawa, Ishibashi, Yoshii, & Tagashira, 1995). However, some researchers have found that muscimol does not effect the retention of radial arm maze tasks (Majchrzak et al., 1990), passive avoidance tasks (Majchrzak et al., 1990), and learning set formation (Bailey & Shutty, 2004; Shutty and Bailey, 2004).

Thus far, there has been no research concerning the possible effect of muscimol on the acquisition of learning set. Based on previous research with acquisition impairments following muscimol injections, the large number of GABAergic neurons located in the nBM, and acquisition impairments following nBM lesions, it was hypothesized that muscimol would impair the acquisition of an odor-discrimination learning set. Animals were tested for learning set using olfactory discriminada, and were tested in the open field to ensure that there were no changes in motor movement or emotionality as a result of the muscimol injections. It was hypothesized that animals receiving muscimol would show deficits in the acquisition of a learning set task, as evidenced by below chance performance on trial 2 across a series of odor-unique discrimination problems.

Methods

Subjects

Fourteen male Long-Evans rats were purchased from Harlan (Indianapolis, IN) and were approximately 120 days old at the beginning of testing. The rats were randomly assigned to one

of two groups: muscimol ($n = 7$) or saline ($n = 7$). The animals were housed in individual metal cages with dimensions of 25.4 cm (length) x 19.8 cm (width) x 17.8 cm (height) and were kept in a temperature-controlled room with a 12:12 light-dark cycle (with the dark cycle beginning at 5 p.m. for the first half of the study, and then 1:30 p.m. for the remainder of the study). All rats were tested during the dark cycle using only a red light to allow for experimenter visibility. The rats were handled extensively during the first four days upon arrival and were handled regularly throughout the remainder of the study. The rats ate *ad libitum* for the first four days upon arriving, but were maintained on three pellets (15g) per day thereafter with water available *ad libitum* for the duration of the study. Animals were weighed daily to ensure health and all animals were treated with above standard care as required by the American Psychological Association (APA) for animals used in psychological research.

Apparatus

Open field. The open field was composed of a gray wooden box that measured 60 cm (length) x 60 cm (width) x 28 cm (height). There were a total of 16 squares painted on the floor of the open field (four inner squares and 12 outer squares). The open field was covered with a see-through wire grid.

Learning set testing chamber. The learning set testing chamber was a polycarbonate plastic cage with measurements of 47 cm (length) x 25 cm (width) x 15 cm (height). At one end of the testing chamber, three clear 3 oz. plastic cups were adhered to a wooden platform with measurements of 9 cm (length) x 18 cm (width) x 1 cm (height). A removable wire grated screen, 34 cm (length) x 21 cm (width), and a wooden block, 30 cm (length) x 18 cm (width) x 2 cm (height), were placed between the rat and the platform to separate the animals from the discriminanda during testing to minimize visual cues during set-up. The testing chamber was also

covered with a wire grated screen which measured 45 cm (length) x 25 cm (width), to prevent the animals from escaping.

Pharmacological Agents

The animals received intraperitoneal (i.p.) injections of either 0.5 mg/kg of muscimol (Sigma, St. Louis, MO) or saline (NLS Animal Health, Owings, MD). The muscimol was dissolved in a 1 mg/ml solution using physiological saline. The pharmacological agents were administered in 1-mL syringes (VWR International, Bridgeport, NJ) with 25 gauge needles (VWR International, Bridgeport, NJ).

Olfactory Discriminada

Twenty-two household spices were used to create the discriminada by adding 2g of spice to 220g of unscented sand. The following spices were used: allspice, black pepper, cayenne, cloves, chervil, cinnamon, cumin, dill, Folgers[®], garlic powder, ginger, Lipton[®], mace, mustard, nutmeg, onion powder, oregano, paprika, rosemary, tarragon, thyme, and turmeric; all of the spices were McCormick[®] brand.

Procedures

Discrimination pre-training. Upon arrival, all rats were handled for four consecutive days. Discrimination pre-training began directly following handling. In the pre-training phase, the rats had to learn to dig for a Froot Loop[®] that was completely buried in a cup of sand. Initially, rats were placed in the testing apparatus and were presented with one cup of unscented sand with three partially buried Froot Loops[®]. The rats were allowed to dig in the cup of sand until all Froot Loops[®] were eaten. The rats repeated this step for a total of three days. On the following day, each rat was presented again with one cup of unscented sand, but with the Froot Loop[®] completely buried under the sand. The rats had to dig for the completely buried Froot

Loop[®] reward and were removed from the testing chamber once they had completed the task 10 times or once 10 minutes had elapsed since the last dig. The rats repeated this step (locating the buried Froot Loop[®]) for a total of four days. The rats were then presented with three cups of unscented sand. One of these cups was selected at random to contain a completely buried Froot Loop[®]. The rats again needed to dig to find the reward and were given 10 total trials per day, and as before, rats were removed from the testing chamber if 10 minutes had elapsed since the last dig. However, in this step the rats were separated from the three cups by a wire grid for the initial 30 seconds of each trial. This step (Froot Loop[®] in one of three cups) was repeated for a total of four days.

Training to criterion. Once each rat had learned to dig for the completely buried Froot Loop[®] on a consistent basis, pre-training to criterion began. Eight household spices were used to create the odor discriminanda in this phase. The spices used were cinnamon, cumin, mustard, thyme, black pepper, nutmeg, onion powder, and tarragon. In this stage, the rat was presented with three 3 oz. clear plastic cups. Two of these cups contained the same scent (non-odd) while the third cup contained a different scent (odd). Once again, the rats were separated from the three cups for the first 30 seconds of each trial to allow the animal to smell each sample before making a choice, and the 10-minute time limit was enforced. The rats completed 10 trials each day and had to dig in the correct cup (odd cup) eight out of 10 times for two consecutive days. Each trial consisted of five correction trials, so the rat could learn over time to dig in the correct (odd) cup. Once the rat had achieved 80% accuracy over two days on the same scent combination, the rat proceeded to the next odor-unique combination. After the rats had been trained to criterion on all four odor combinations, they moved on to olfactory discrimination learning set testing.

Olfactory discrimination learning set (ODLS) testing. All 22 household spices were used as olfactory discriminanda in this phase. The rats were presented with three 3 oz. clear plastic cups, each filled with scented sand. Two cups were filled with the same scent (non-odd), while the third cup was filled with a different scent (odd). The rat had to dig in the correct (odd) cup to gain the Froot Loop[®] reward. As before, the animals were separated from the odor discriminanda by a wire grid screen for the first 30 seconds of each trial, and the 10-minute limit was still enforced. Initially, the animals were given two problems per day for the first 10 problems, and then three problems per day for the remaining 30 problems. Each problem consisted of five trials, and the rats completed a total of 40 odor-unique discrimination problems, while either receiving muscimol or saline. The rats were randomly assigned to one of two groups—one group received muscimol (0.5 mg/kg; i.p.) during this phase and the other group received comparable amounts of saline (i.p.) approximately 15 minutes prior to testing. Following the completion of the original 40 problems, the animals were given an additional 30 problems with no injections.

Open field testing. Rats were habituated to the open field within their first four days of arrival. The rats were then tested in the open field during discrimination pre-training as a pre-treatment measure, after the completion of the initial 40 odor unique discrimination problems as a treatment (muscimol or saline) measure, and after completion of the last 30 problems as a post-treatment measure. Each rat was placed in the lower left corner of the open field and each square entered with all four feet was recorded for five minutes. The number of outer and inner squares entered was also recorded, as well as the number of times the rat engaged in rearing, grooming, and freezing behaviors. Finally, the number of fecal boli was also recorded as a measure of emotionality.

Results

Training to Criterion

An independent t-test was used to analyze any differences in muscimol and saline treated rats during the training to criterion phase. The muscimol-treated animals ($M = 105.71$, $SD = 15.12$) did not differ significantly from the saline-treated animals ($M = 101.43$, $SD = 18.64$) in the number of trials taken to successfully train for the oddity discrimination task, $t(12) = -0.472$, $p = 0.645$. This result suggested that the saline- and muscimol-treated rats did not differ significantly prior to injections, since all animals required approximately the same number of trials to reach criterion. Furthermore, the saline- ($M = 22.14$, $SD = 13.36$) and muscimol-treated rats ($M = 32.00$, $SD = 19.22$) did not differ significantly in the number of errors committed during these training problems, also suggesting that the two groups did not initially differ in their abilities to perform simple discrimination tasks, $t(12) = -1.11$, $p = .29$.

Acquisition of Learning Set

Binomial approximations were used in order to determine whether the saline- and muscimol-treated rats performed significantly different from chance across the original 40 problems. Although there were three positions, chance was considered to be 50% as a more conservative value since there were only two actual odor choices. Overall performance on the odor discrimination task was examined by observing each group's performance across all five trials on the original 40 odor-unique discrimination problems (see Figure 1). On Trial 1, saline-treated rats performed at levels that did not differ significantly from chance ($p = .5$) and muscimol-treated rats performed at levels significantly lower than expected by chance ($p < .05$, see Figure 1). On Trials 2-5, all animals performed significantly higher than expected by chance

(all p 's < .05, see Figure 1), with the exception of two animals receiving saline and one animal receiving muscimol (see Table 1).

A two-way mixed Analysis of Variance (ANOVA) (Trial x Treatment) was used to determine whether any differences existed between the treatment groups on Trials 1-5 of the original 40 problems. There was a significant effect of Trial ($F(4, 48) = 21.77, p < .001$), indicating a general increase in performance after Trial 1 (see Figure 1). Post-hoc paired sample t-tests with Bonferroni corrections (new $\alpha = .005$) were used to determine the trials that differed significantly from one another. Significant differences were found between Trial 1 ($M = 49.96, SD = 8.27$) and all other trials (all p 's < 0.005) and between Trial 3 ($M = 61.19, SD = 12.57$) and Trial 5 ($M = 71.00, SD = 11.90$), $p < .005$. No other significant differences were found. There was no significant differences in performance found between saline- ($M = 63.98, SE = 3.71$) and muscimol-treated rats ($M = 59.64, SE = 3.705$), $F(1, 12) = 0.69, p = .42$. In addition, there was no significant interaction between Trial and Drug Treatment, $F(4, 48) = 2.07, p = 0.099$.

In order to examine any changes in Trial 2 performance across the original 40 odor-unique discrimination problems, these 40 problems were divided into blocks of 10 problems. Binomial approximations were used to see if the animals performed at levels that differed significantly from chance. Muscimol- and saline-treated rats performed significantly above chance on Blocks 3 and 4 (all p 's < .05), indicating that they successfully formed and used a learning set on problems 21-40 (see Figure 2). A two-way mixed ANOVA (Block x Treatment) revealed a significant effect of Block, $F(3, 36) = 13.68, p < .001$. Paired sample post-hoc t-tests with Bonferroni corrections (new $\alpha = .008$) were used to determine which blocks differed significantly from one another. There were significant differences between Block 1 ($M = 54.90, SD = 14.59$) and Block 3 ($M = 73.57, SD = 19.46$) ($t(13) = -4.24, p < .008$), between Block 1

and Block 4 ($M = 71.43$, $SD = 15.12$) ($t(13) = -3.37$, $p < .008$), Block 2 ($M = 48.57$, $SD = 20.70$) and Block 3 ($t(13) = -5.83$, $p < .008$), and between Block 2 and Block 4 ($t(13) = -4.95$, $p < .008$). No other significant differences were found. There was no significant interaction ($F(3, 36) = .24$, $p = .87$) nor were there any significant differences between muscimol- ($M = 61.61$, $SE = 5.60$) and saline-treated rats ($M = 62.63$, $SE = 5.60$), $F(1, 12) = .017$, $p = .90$.

Post-Injection Performance

A two-way mixed ANOVA (Time of Measurement x Treatment) was used to determine whether the animals differed on Trial 2 performance between injections and post injections. There was a significant effect of the time of measurement, with rats performing significantly higher on Trial 2 during post-injection problems ($M = 80.16$, $SE = 3.23$) than while receiving injections ($M = 62.21$, $SE = 3.99$), $F(1, 12) = 23.08$, $p < .001$. There was no significant interaction, $F(1, 12) = .27$, $p = .61$, nor was there any difference found between the saline- ($M = 72.76$, $SE = 4.39$) and muscimol-treated rats ($M = 69.61$, $SE = 4.39$), $F(1, 12) = .26$, $p = .62$ (see Figure 3).

Binomial approximations were used to examine overall performance on Trials 1-5 across the 30 post-injection problems. The binomial approximations indicated that all rats performed significantly higher than expected by chance on Trials 1-5 of the post-injection phase (all p 's $< .05$, see Figure 4). A two-way mixed ANOVA (Trial x Treatment) was also used to determine whether the rats differed significantly across all five trials of the post-injection phase. A significant difference was found in performance across all five trials, $F(4, 48) = 14.82$, $p < .001$. Post hoc paired sample t-tests with Bonferroni corrections (new $\alpha = .005$) were used to determine which trials differed from one another. These analyses revealed that the rats performed significantly worse on Trial 1 ($M = 63.33$, $SD = 12.60$) than on Trials 2-5. Rats performed

significantly worse on Trial 1 than on Trial 2 ($M = 80.16$, $SD = 11.89$), $t(13) = -3.94$, $p < .005$). Rats also performed significantly worse on Trial 1 than on Trial 3 ($M = 78.17$, $SD = 11.56$), $t(13) = -3.66$, $p < .005$; significantly lower on Trial 1 than on Trial 4 ($M = 81.65$, $SD = 8.71$), $t(13) = -5.73$, $p < .005$; and significantly lower on Trial 1 than on Trial 5 ($M = 85.90$, $SD = 9.10$), $t(13) = -5.22$, $p < .005$. There was no interaction between performance across all five trials and the treatment ($F(4, 48) = 1.35$, $p = .27$), nor was there a significant difference in performance between the saline- ($M = 80.53$, $SE = 2.87$) and muscimol-treated rats ($M = 75.15$, $SE = 2.87$), $F(1, 12) = 1.76$, $p = .21$.

Open Field Testing

A three-way mixed ANOVA (Time of Measurement x Type of Square x Treatment) was performed to analyze any differences in the number of outer and inner squares entered. Rats entered significantly more outer squares ($M = 88.88$, $SE = 4.63$) than inner squares ($M = 16.02$, $SE = 2.04$), $F(1, 12) = 352.93$, $p < .001$ (see Figure 5). There was no significant effect of the Time of Measurement ($F(2, 24) = 2.797$, $p = .08$), nor was there any significant effect of the Treatment ($F(1, 12) = .001$, $p = .98$). There were no significant interactions between Type of Square and Treatment ($F(1, 12) = 1.14$, $p = .31$), between Time of Measurement and Treatment ($F(2, 24) = 1.24$, $p = .31$), nor between Treatment, Type of Square, and Time of Measurement ($F(2, 24) = 1.29$, $p = .30$).

A two-way mixed ANOVA (Time of Measurement x Treatment) was used to analyze the total number of squares entered. There was no significant effect of time of measurement (pre-injection, injection, post-injection) for the number of Total Squares Entered ($F(2, 24) = 2.80$, $p = .08$), no significant effect of the Treatment ($F(1, 12) = .001$, $p = .98$), or any significant

interaction between time of measurement and the treatment ($F(2, 24) = 1.24, p = .31$), see Figure 6.

Two-way mixed ANOVA's (time of measurement x treatment) were also used to analyze the remaining open field behaviors. There was no significant effect of Time of Measurement for rearing behavior ($F(2, 24) = 1.73, p = .20$), no significant effect of the Treatment on rearing behavior ($F(1, 12) = 1.13, p = .31$), or any significant interactions between the Time of Measurement and Treatment ($F(2, 24) = .36, p = .70$), see Figure 7. There was no significant effect of Time of Measurement on grooming behavior ($F(2, 24) = 3.22, p = .06$), no significant effect of Treatment ($F(1, 12) = .02, p = .89$), or any significant interaction between Time of Measurement and Treatment ($F(2, 24) = .48, p = .62$), see Figure 8. In addition, there was no significant effect of Time of Measurement on freezing behavior ($F(2, 24) = .80, p = .46$), no significant effect of the Treatment ($F(1, 12) = 1.84, p = .20$), or any significant interactions between Time of Measurement and Treatment ($F(2, 24) = .80, p = .46$), see Figure 9. Finally, there was no significant effect of Time of Measurement on the number of fecal boli ($F(2, 24) = 1.28, p = .30$), no significant effect of Treatment ($F(1, 12) = .14, p = .72$), or any significant interaction between the Time of Measurement and the Treatment ($F(2, 24) = .72, p = .50$), see Figure 10.

Discussion

No significant differences in learning set performance, as measured by Trial 2 performance, were found upon treatment with muscimol as hypothesized. The majority of the animals were able to form and use a learning set regardless of the type of injection received, as indicated by the significantly higher than chance performance on Trial 2 across a series of odor-unique discrimination problems. Only three animals did not demonstrate evidence of learning

set formation. Two of these animals received saline and one received muscimol, so it is unlikely that these impairments were a result of treatment with muscimol. Although muscimol did not appear to block the acquisition of learning set, it could be hypothesized that muscimol may produce a significant impairment (i.e. significantly lower performance when compared to saline controls) in learning set performance. However, this did not appear to be the case either, since there were no overall significant differences between the muscimol- and saline-treated rats. This suggests that muscimol did not produce a significant impairment in the rats' abilities to form and use a learning set.

Furthermore, the rats performed in accordance with previous trends recognized by other researchers (Bailey & Shutty, 2004; Bailey & Thomas, 2001; Lee, Grabill, & Bailey, 2003; Martin, 2003; Shutty & Bailey, 2004). Specifically, the rats began to display evidence of learning set, as evidenced by performances higher than expected by chance, beginning with problem 21 and continuing through problem 40 (Blocks 3 and 4). This trend was recognized regardless of pharmacological treatment, suggesting that muscimol did not interfere with the animals' ability to acquire a learning set. Additionally, the animals sustained the above chance performance throughout the rest of the testing phase, with no significant differences occurring between the saline- and muscimol-treated rats.

Some may argue that the muscimol-treated rats did not show any impairment in the acquisition of learning set, due to the possibility of that group of rats performing at a higher baseline level compared to the saline-treated rats. However, this was not the case, as both the saline- and muscimol-treated rats required a similar number of trials to reach criterion in the training phase and did not differ significantly in the number of trials to reach criterion or in the

number of errors committed during these training problems. Thus, this established that both groups of rats did not differ significantly at the start of the investigation.

In addition, both saline- and muscimol-treated rats tended to improve once injections were no longer being delivered, as evidenced by significantly improved performance between the injection phase and the post-injection phase. However, there were no significant differences between the two groups, suggesting that there were no underlying learning differences as a result of muscimol. Rather, this improvement was more than likely due to practice effects. The animals probably improved merely because they had more exposure to the task and strengthened understanding of the learning set hypothesis.

In accordance with previous research, the dose of 0.5 mg/kg did not appear to impair motility in any way, due to the saline- and muscimol-treated rats tending to perform at comparable levels in the open field (Shutty & Bailey, 2004). There were no significant differences between the two groups in the total number of squares entered, the types of squares entered, or in rearing, grooming, and freezing behaviors. In addition, the decrease in rearing previously reported by Bailey & Shutty (2004) with 0.5 mg/kg of muscimol was not confirmed in this investigation. Furthermore, this dose of muscimol did not appear to affect emotionality, as measured by the number of fecal boli deposited in the open field; both groups of rats tended to defecate at approximately the same rates.

A 0.5 mg/kg dose of muscimol was chosen as a result of findings from previous research. Oksztel and Wisniewski (2002) found that muscimol doses of 1.0 mg/kg resulted in decreased motility when tested in the open field, and Houston, Wong, and Ebenezer (2002) found that muscimol doses of 1.0-2.0 mg/kg resulted in significantly lower levels of water intake, while 0.5 mg/kg did not produce this effect. As a result, a dose of 0.5 mg/kg was chosen for this

investigation. Perhaps, this conservative dose of 0.5 mg/kg was too low to exhibit any impairment in learning set performance. Higher doses should be tested to determine the maximum dose that can be used without impairing motility, but which may impair the acquisition of learning set. Furthermore, previous studies infused muscimol directly into the nBM and found significant impairments 30 minutes post-infusion (Cooke, Attwell, & Yeo, 2004). It is possible that the 15-minute wait time that was allowed after the injections was not long enough to warrant any visible impairment, especially since the muscimol was delivered via an intraperitoneal injection, and was not infused directly into the area of interest. Therefore, future investigations into the role of the GABAergic system in learning set formation should make use of direct injections into the nBM and perhaps allow a longer wait time before the testing session.

Due to previous investigations with the GABAergic system, it is very likely that the GABAergic system plays some role in the acquisition of learning set. Numerous studies have shown that muscimol can impair performance in various learning paradigms, such as retention of inhibitory avoidance (Castellano & McGaugh, 1990; Morón et al., 2002), retention of latent inhibition (Holt & Maren, 1999), retention of reward memory (Salinas & McGaugh, 1995), and place learning in the water maze (Nakagawa et al., 1995). Muscimol has also been shown to affect acquisition in taste and place aversive learning (Morón et al., 2002), radial arm maze (Majchrzak et al., 1990), passive avoidance (Majchrzak et al., 1990), and fear conditioning (Muller et al., 1997). Due to the ability of muscimol to affect both retention and acquisition in numerous learning paradigms, it would seem likely that it would affect the acquisition of a learning set in the same way. However, learning set is relatively different from these other learning paradigms (specifically passive avoidance, taste and place aversive learning, and fear

conditioning) because olfactory-discrimination learning set testing involves a choice between olfactory discriminanda. Furthermore, learning set is a much more cognitively demanding task, and may use different neurological circuits for learning. Perhaps, muscimol does not exert its effects on the ability of rats to differentiate correctly between odiferous stimuli.

Several problems were encountered throughout this research project that may have contributed to the observed results. Many difficulties were encountered during the training and testing processes. Specifically due to time constraints, every animal could not be tested every day. Furthermore, there were difficulties controlling the air temperature in the housing room, such that the animals were slightly overheated for a portion of the study. This abundance of heat resulted in the animals being very lethargic during the training phase and taking much longer to complete the training phase than in previous investigations. It is always possible that these difficulties interfered with the testing process enough to conceal any significant impairment that would have normally been detected. Therefore, in order to clarify the possible involvement of the GABAergic system on learning set performance, more research needs to be performed, investigating higher doses of GABAergic agents and infusing these agents directly into the area of interest—in this case, the nBM—while also ensuring that the temperature stays constant and the animals are tested every day. With the use of these additional controls, it is possible that there is in fact an involvement of the GABAergic system in the acquisition of a learning set.

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Table 1.

Mean Percent Correct for Each Rat during the Initial 40 Odor-Unique Discrimination Problems

Rat	Treatment	Mean Percent Correct				
		Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
600	saline	47.40	64.90	61.10	60.00	82.90
601	muscimol	37.50	75.00	65.00	72.50	72.50
602	muscimol	42.50	57.50	40.00	57.50	50.00
603	saline	70.00	87.20	73.00	86.10	88.90
604*	saline	37.50	37.50	55.00	41.20	56.40
605	saline	52.50	64.10	63.20	70.30	67.60
606	saline	40.00	69.20	63.90	60.00	70.60
607	muscimol	45.00	76.30	81.60	81.60	73.70
608	muscimol	45.00	60.00	42.50	65.00	65.00
609	muscimol	47.50	55.00	66.70	73.70	71.10
610	muscimol	45.00	70.00	67.50	75.70	59.50
611*	muscimol	45.00	37.50	42.50	60.00	65.00
612*	saline	47.50	47.50	59.00	68.40	78.90
613	saline	55.00	69.20	75.70	75.70	91.90

*These rats did not successfully acquire a learning set, as evidenced by below chance (50%) performance on Trial 2.

Figure Captions

Figure 1. The mean percent correct ($\pm SD$) across all five trials during the initial 40 odor-unique discrimination problems for saline- and muscimol-treated rats.

Figure 2. The mean percent correct on Trial 2 ($\pm SD$) across blocks of 10 problems during the acquisition phase for saline- and muscimol-treated rats.

Figure 3. The mean percent correct on Trial 2 ($\pm SD$) during the injection phase and post-injections for saline- and muscimol-treated rats.

Figure 4. The mean percent correct ($\pm SD$) across all five trials during the final 30 odor-unique discrimination problems for saline- and muscimol-treated rats.

Figure 5. The mean number of outer and inner squares ($\pm SD$) entered at each of the three measurement times for saline- and muscimol-treated rats.

Figure 6. The mean number of total squares ($\pm SD$) entered during each of three measurement times for saline- and muscimol-treated rats.

Figure 7. The mean number of times ($\pm SD$) the saline- and muscimol-treated rats engaged in rearing behaviors during the three measurement times.

Figure 8. The mean number of times ($\pm SD$) the saline- and muscimol-treated rats engaged in grooming behaviors in the open field during each of the three measurement times.

Figure 9. The mean number of times ($\pm SD$) the saline- and muscimol-treated rats engaged in freezing behaviors in the open field at each of the three measurement times.

Figure 10. The mean number of fecal boli ($\pm SD$) deposited in the open field by saline- and muscimol-treated rats at each of the three measurement times.

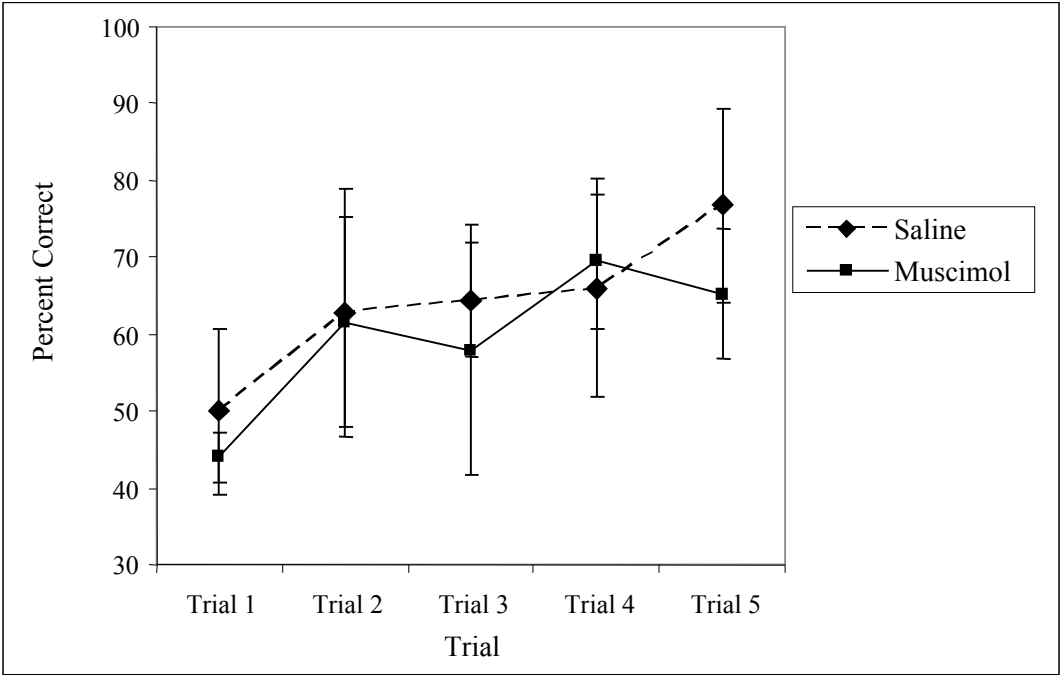


Figure 1

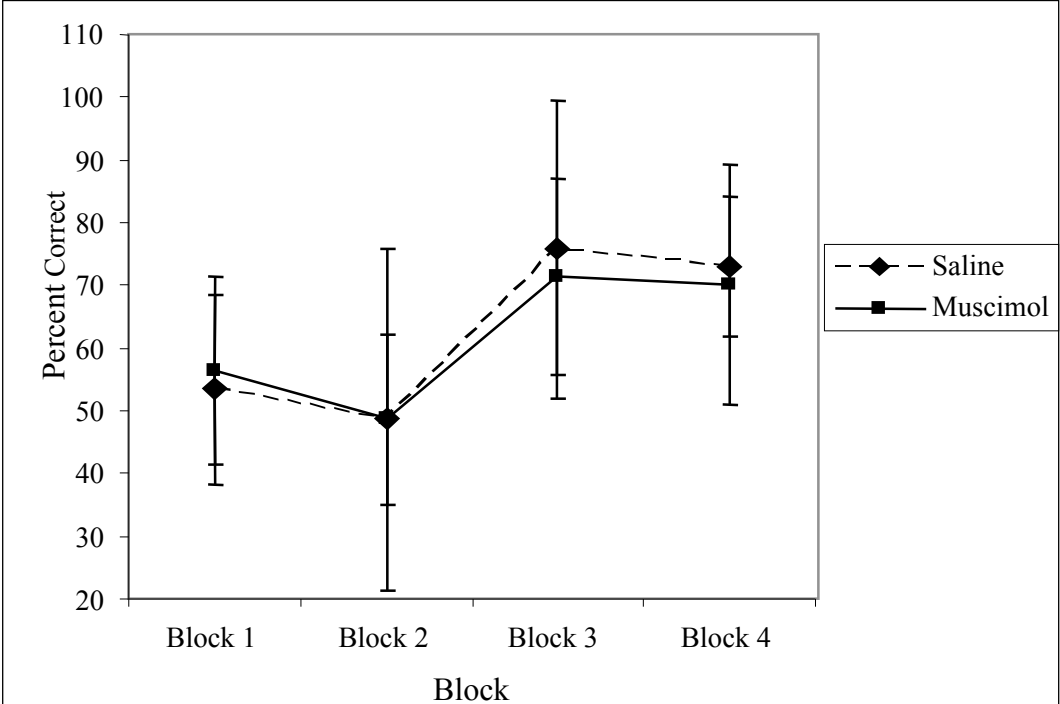


Figure 2

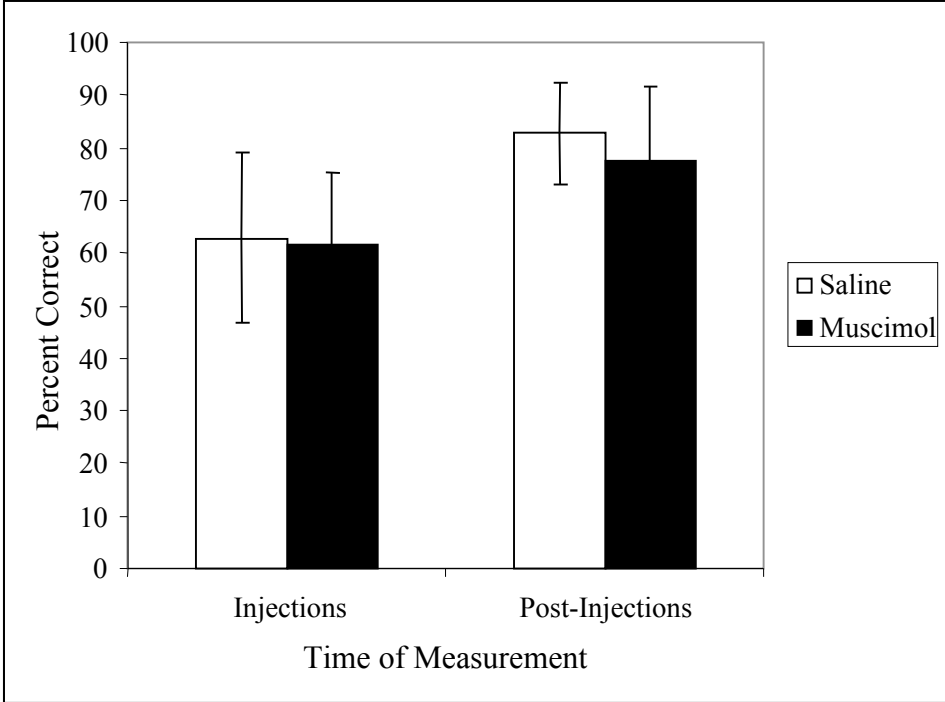


Figure 3

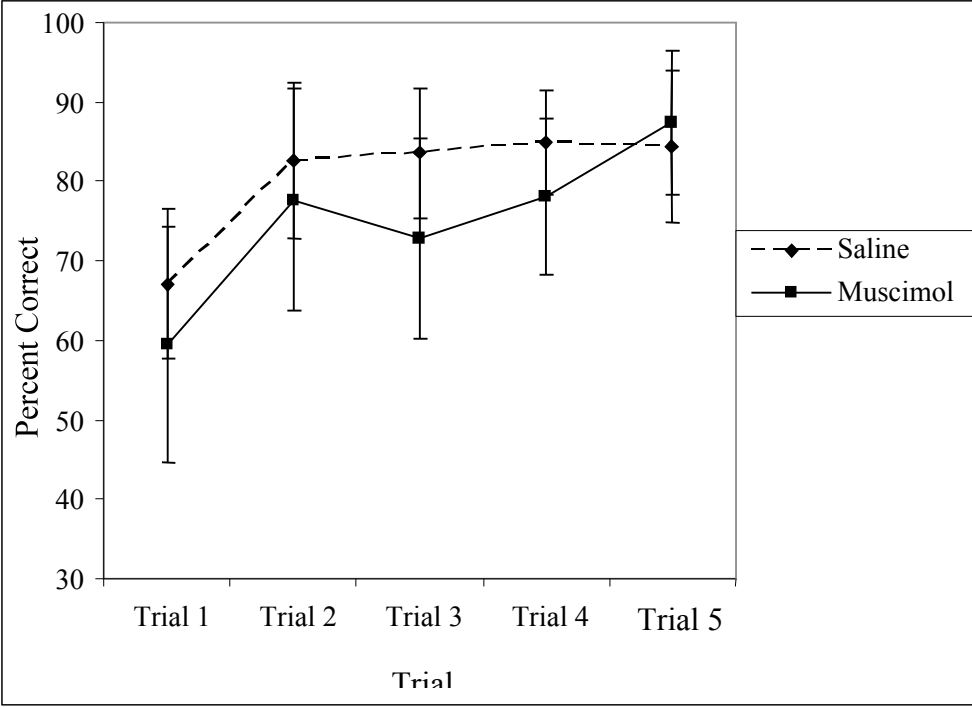


Figure 4

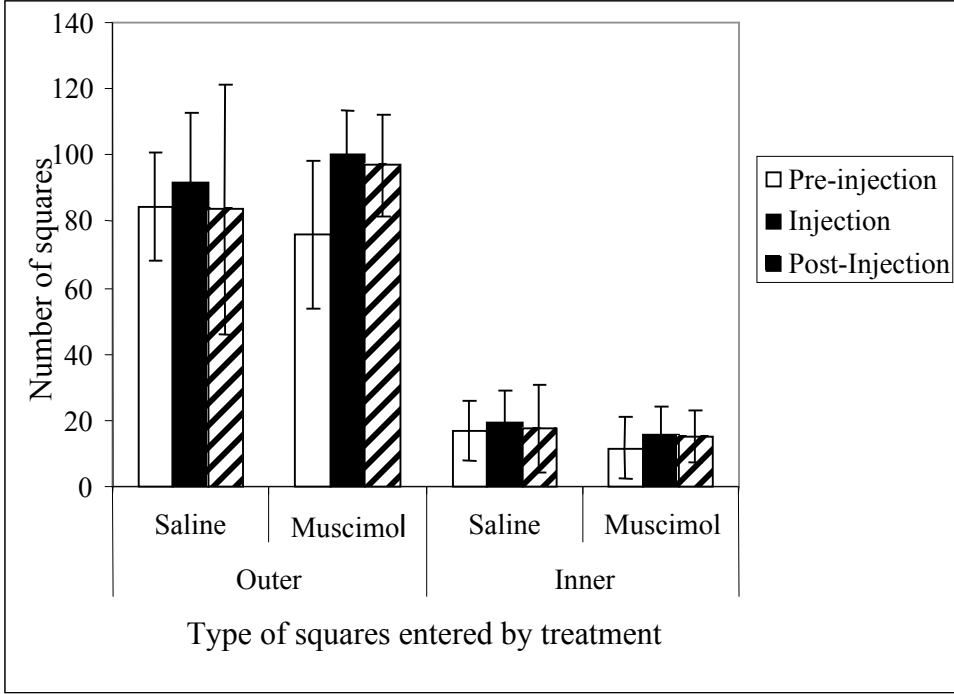


Figure 5

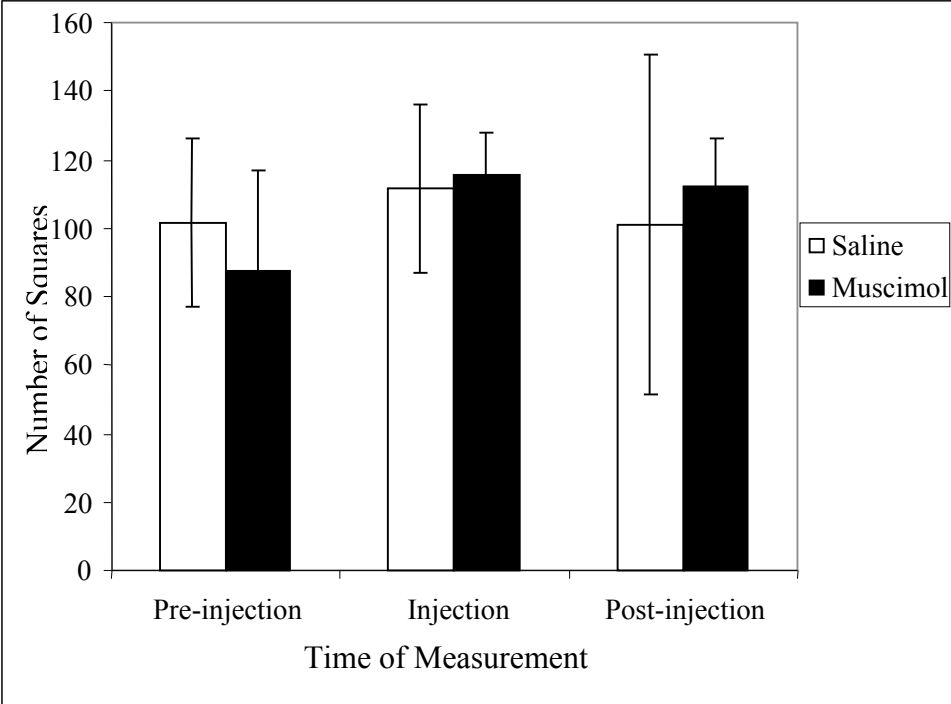


Figure 6

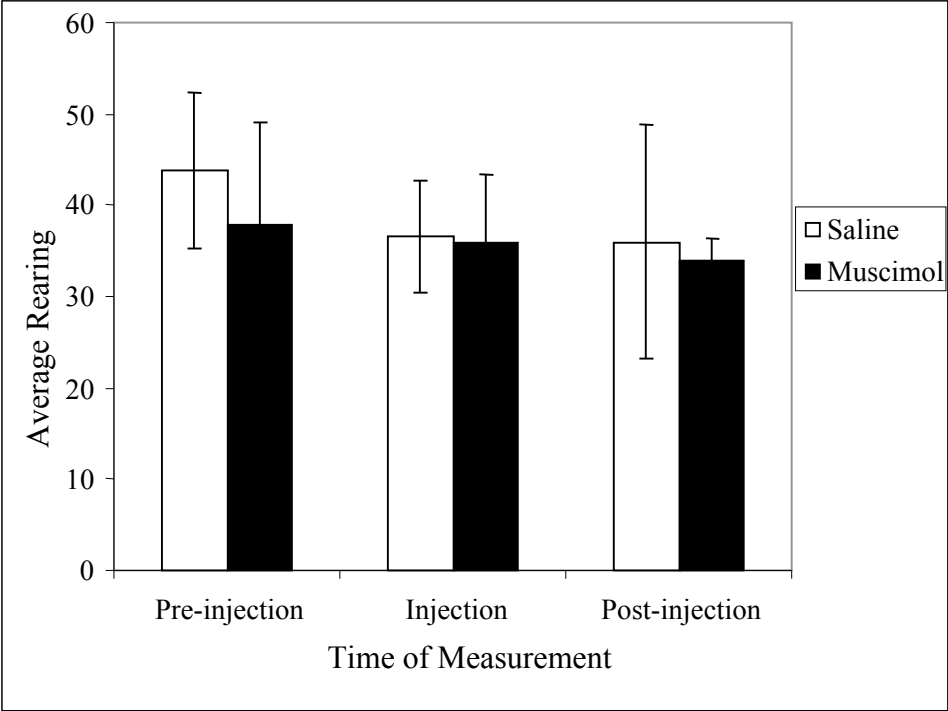


Figure 7

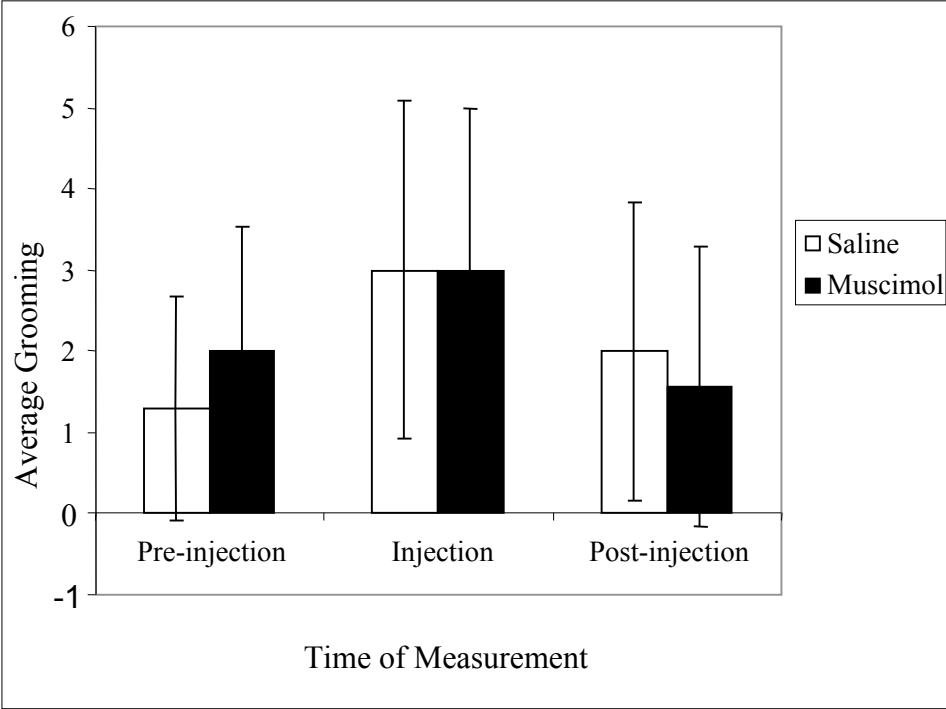


Figure 8

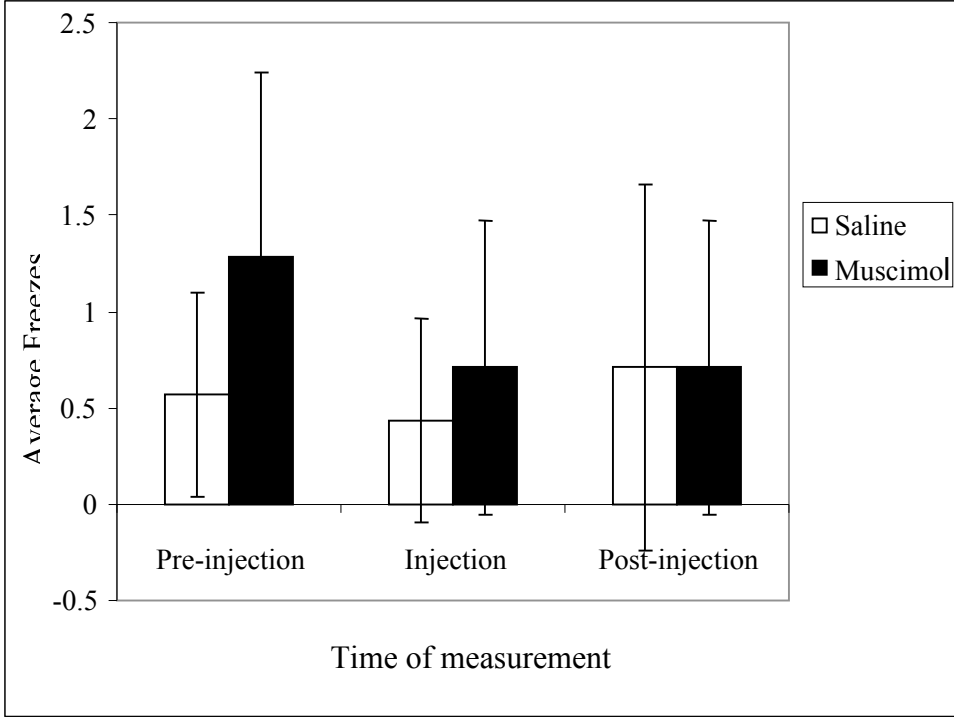


Figure 9

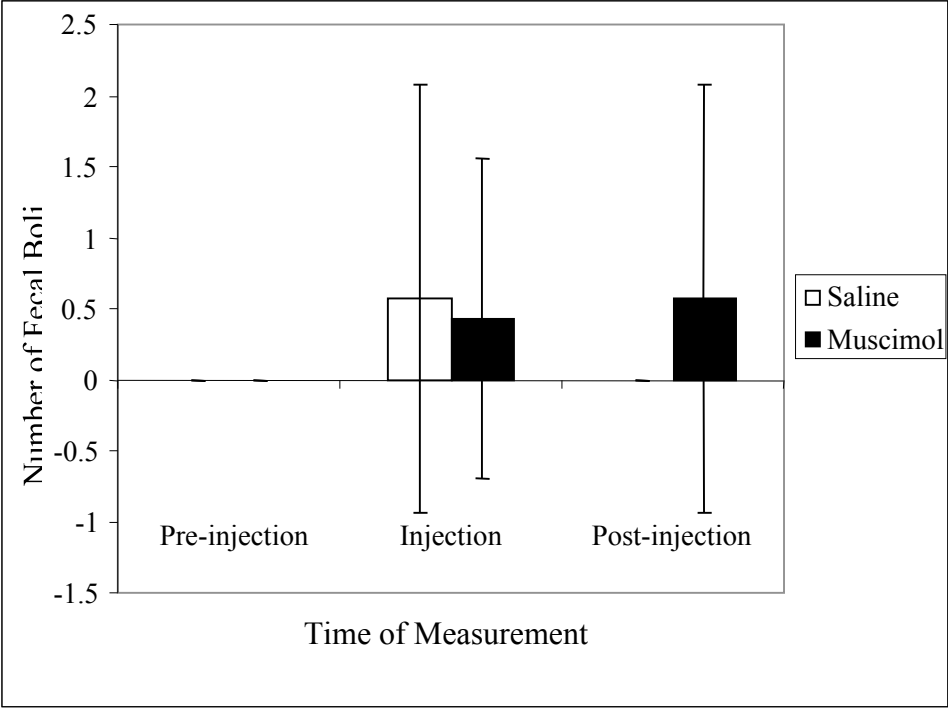


Figure 10

Appendix

Alzheimer's Disease

Alzheimer's disease (AD) is a progressive degenerative disorder of the brain, which gradually robs people of important life processes, including memory, awareness, and eventually the ability to control bodily functions, resulting in death (Papalia, Sterns, Feldman, & Camp, 2002). Patients gradually lose the ability to learn new information and eventually lose the ability to recall previously learned information (Bear, Connors, & Paradiso, 2001). As of 2002, AD was the fourth leading cause of death in the United States and approximately 4 million people in the United States had been diagnosed (Papalia et al., 2002). The disease was first characterized by Alois Alzheimer in 1907 when he examined a patient's brain and found extensive loss of nerve cells and a shrinkage of the cerebral cortex, as well as large masses of amyloid plaques and neurofibrillary tangles (Papalia et al., 2002).

The two forms of AD, early-onset and late-onset, differ in the genetic causes as well as in the symptoms and age of onset. The symptoms of early-onset AD usually appear in the patient before ages 60 to 65, and often before 55 years of age. Early-onset AD seems to be caused by mutant genes on chromosomes 14 and 21 (Karlinsky, Lennox, & Rossor, 1994), and is usually marked by more severe symptoms, with the progression of the disease usually occurring more quickly. The symptoms of late-onset AD usually appear in the patient at a later age and may also be genetically inherited through a pair of genes on chromosome 19 (Roses, 1994).

AD is characterized by a marked deterioration in cholinergic neurons in the brain, mainly in the medial septal area, hippocampus, neocortex, and the nucleus of Meynert (the nucleus basalis magnocellularis) (Bear et al., 2001). The medial septal area and the nucleus of Meynert are in the basal forebrain and provide cholinergic innervation to the hippocampus and neocortex,

respectively (Bear et al., 2001). The structures of the basal forebrain have been implicated in several aspects of learning and memory, including taste and place aversive memory formation (Morón et al., 2002), learning set formation (Bailey & Thomas, 2001), and radial arm maze performance (Wellman & Pelley, 1999).

AD is associated with an abnormal secretion of a naturally occurring protein, amyloid, which appears to be the first step in the neurotoxicity that leads to the formation of the plaques and tangles evident in AD (Doody, 1999). The amyloid plaques form when amyloid precursor protein is cleaved improperly, resulting in sticky amyloid that then aggregates to form the plaques. These plaques result in interference of nerve cell communication, so that messages are not received properly (Papalia et al., 2002). Mouse models of AD have shown that the amyloid plaques tend to appear mainly in the hippocampus and neocortex (Bondolfi et al., 2002), following the localization that also occurs in AD patients (Nagy, Esiri, Jobst, Morris et al., 1996).

In the neurons that are destroyed in AD, there is also a change in the neurofibrils of the cytoskeleton, so that they become thicker and accumulate to form the dense bundles known as neurofibrillary tangles (Bear et al., 2001). Eventually, the tangles impede the flow of information between neurons. As a result of this lack of communication, the neurons die and their death is marked by the disappearance of the nucleus and cytoplasm, leaving only the neurofibrillary tangles (Bear et al., 2001). The neurofibrillary tangles are a result of the malfunction of a naturally occurring protein, tau. Tau normally functions to ensure that microtubules run straight and parallel to each other. However, in AD, tau does not anchor properly to the microtubules and instead accumulates in the soma, causing the axons to wither and a blockage of normal information flow between neurons occurs (Bear et al., 2001). Nagy,

Esiri, Jobst, & Morris (1995) found that the cognitive deficit in AD patients was highly correlated with neurofibrillary tangle density in the frontal and parietal lobes of the brain. Furthermore, the relative densities of both amyloid plaques and neurofibrillary tangles were positively associated with the duration of AD in the tested patients (Nagy et al., 1995).

In addition to the presence of amyloid plaques and neurofibrillary tangles, AD is also associated with the degeneration of hippocampal neurons and a general atrophy of the cerebral cortical sulci (Reisine, Yamamura, Bird, Spokes, & Enna, 1978). AD is also associated with a depletion of the neurotransmitter acetylcholine (due to loss of acetylcholine-producing neurons), loss of muscarinic receptors, and a decline in choline acetyltransferase (ChAT) activity (“Challenging the Cholinergic Hypothesis,” 2004). The decrease in ChAT activity has been found specifically within the cerebral cortex and the nucleus basalis of Meynert (Liberini, 1997), as well as in the frontal cortex, temporal cortex, hippocampus, and cerebellum (Heo et al., 2003). Levels of ChAT and acetylcholinesterase in the brains of AD patients can be decreased by as much as 90% compared to controls (Giacobini, 2003). Furthermore, the decrease in ChAT activity in AD patients is related to the degree of dementia and the severity of neuropathological hallmarks of the disease, such as amyloid plaques and neurofibrillary tangles (Heo et al., 2003). Additionally, Park, Pappas, Murtha, and Ally (1992) found that weanling rats which were raised in an enriched environment had higher levels of ChAT in the caudate than impoverished rats. The researchers also found that the higher levels of ChAT correlated with increased learning of the Morris water maze task.

AD has also been associated with a decrease in other compounds, such as norepinephrine, in the nucleus caudatus, putamen, and globus pallidus (Nyberg et al., 1985). However, there appear to be no deficiencies of norepinephrine in the hippocampus of AD brains (Nyberg et al.,

1985). Further studies revealed that the levels of ChAT and noradrenaline were more severely depleted in early onset AD patients than in the late-onset AD group (Arai et al., 1992).

Nucleus Basalis Magnocellularis (nBM)

The nBM is located in the caudal region of the basal forebrain, and it directly parallels the human nucleus basalis of Meynert (Fibiger, 1982; Manfredi, Brambilla, & Mancina, 2001).

The nBM has been investigated extensively in learning and memory, as neuronal loss in the nBM is prevalent in AD and the neuronal loss in the nBM may be responsible for the observed cognitive deficits (Bear et al., 2001). Aged animals have also shown decreases in the size and density of functional nBM neurons, which corresponds with cognitive impairment seen in aging (Reisine et al., 1978). The nBM contains cholinergic neurons, as well as GABAergic interneurons, and receives inhibitory GABAergic projections from the nucleus accumbens (Morón et al., 2002). Majchrzak, Brailowsky, & Will (1990) observed a dose-dependant gliosis of the nBM with GABA treatment that could be prevented by saline pretreatment, suggesting that GABA may be involved in the proliferation of supporting tissue in the nBM.

nBM lesions in the past have been used as an attempt to model the changes observed with AD. Connor, Langlais, and Thal (1991) lesioned the nBM with either ibotenic acid or quisqualic acid in F344 rats and analyzed the nBM-cortical pathway for levels of ChAT. Quisqualic acid resulted in a slightly higher decrease in ChAT activity in both the anterior and posterior regions of cortex than did ibotenic acid. Both quisqualic acid and ibotenic acid lesions resulted in the rats taking significantly longer to find the hidden platform in a Morris water maze task. In addition, the swim speed and open field behavior of the rats were examined to determine if these lesions had any effects on motility; these analyses did not show significant differences in general motor activity. Other cortical amino acid levels were examined to see if these neurotoxins had

any effect on any other systems. The researchers found that [3H]neurotensin binding, dopamine, norepinephrine, and serotonin levels were not affected as a result of either neurotoxin. A histological examination also revealed that ibotenic acid resulted in greater damage to non-nBM structures than quisqualic acid, including the basolateral nucleus of the amygdala and the reticular formation of the thalamus. Due to its ability to reduce the levels of ChAT, ibotenic acid has been used to produce an animal model of AD, since levels of ChAT are depleted in AD patients.

Previous research with animal models of AD have lesioned the nBM, because it parallels the nucleus basalis of Meynert in humans. Furthermore, the lesions have been non-specific, as well as specific for cholinergic neurons. nBM lesions in the past have been shown to produce deficits in various learning and memory tasks such as radial arm maze performance. Wellman and Pellemounter (1999) investigated the effect of ibotenic acid lesions of the nBM on radial arm maze performance in young, middle-aged, and aged rats. The researchers found that the middle-aged and aged rats with lesions demonstrated significantly lower performance levels, as evidenced by the rats requiring more trials to reach criterion and committing more errors than either the sham or control (non-operated) rats. Although the lesions produced deficits across all ages, both the middle-aged and aged rats showed more profound deficits in the radial arm maze task. Therefore, both nBM lesions and old age appear to impair radial arm maze performance similarly in rats, thus making nBM lesions a good model for AD or other age-associated dementia.

Due to previous findings, nBM-lesioned animals have been used successfully as a model of AD. Both specific and non-specific cholinergic lesions are used depending on the desired result. Research on learning set formation is among the research utilizing nBM-lesioned

animals. The research on learning set formation is designed to further investigate the neurochemical characteristics of AD.

Learning Set Formation

Nonspecific neurotoxic lesions to the nBM have been shown to significantly impair learning set formation (Bailey & Thomas, 2001). Learning set has been defined as “learning how to learn efficiently in a situation an animal frequently encounters” (Harlow, 1949, p. 51) or as a win-stay/lose-shift hypothesis (Levine, 1965). This hypothesis predicts that if an animal is presented with two objects and it responds to the correct object on Trial 1 and is rewarded, it should stay with this choice on subsequent trials. However, if the animal chooses incorrectly on Trial 1, it should learn to shift its response to the other choice on subsequent trials. Therefore, it can be said that the animal has successfully formed a learning set if it performs significantly above chance (50%) on Trial 2 on a set of discrimination problems.

The ability to acquire a learning set has most commonly been associated with primates, and has been used to rank organisms for intelligence (Slotnick & Katz, 1974). Initial studies with rhesus monkeys indicated that the acquisition of a learning set depended only on the number of trials given, and did not depend on how these trials were organized into problems (Levine & Harlow, 1959). In other words, the ability of an animal to acquire a learning set did not depend on the types of stimuli used or the order in which these stimulus combinations were presented; rather, it depended on how many problems the animals needed to complete.

Slotnick and Katz (1974) tested the ability of rats to acquire an olfactory learning set using scented air puffs. The researchers found that the animals' performance improved rapidly within problems, as well as across a series of problems. The animals' performance suggested they were utilizing a “win-stay/lose-shift” hypothesis, due to the animals' tendency to inhibit

responding to further presentations of a stimulus, which was not reinforced. The researchers also observed three distinct patterns of behavior during the training phase: 1) odor sampling during the first four to eight trials, 2) little or no odor sampling after several problems due to rapid key responses once the trial was initiated, and 3) improvement in performance correlating with the animals' tendency to return to the odor sampling technique. One-trial learning was also associated with the animal engaging in initial odor sampling. These results demonstrated that rats have the ability to rapidly acquire a learning set for odor stimuli that is comparable to the learning sets achieved by primates in response to visual stimuli. Furthermore, Lovelace and Slotnick (1995) tested rats on 16 novel 2-odor discrimination tasks and discovered that rats were able to remember for 30 minutes whether a single brief presentation of an odor was followed by a reward, suggesting that the animals had acquired a learning set and were able to keep this information in memory for 30 minutes after the stimulus presentation.

Other researchers have tested the ability of rats to acquire an olfactory discrimination learning set using scented sand (Bailey & Shutty, 2004; Lee et al., 2003; Martin, 2003; Shutty & Bailey, 2004). These researchers still used two olfactory discriminanda (two scents), but instead, used three cups of scented sand. The animals had to choose the correct scent (the scent which was only represented once), and if the correct scent was chosen, the rats were allowed to dig for the Froot Loop® reward.

Bailey and Thomas (2001) tested the ability of nBM-lesioned rats to acquire a learning set. Quisqualic acid, a nonselective neurotoxin, was used to lesion the nBM and rats were tested on an olfactory discrimination task. The nBM-lesioned rats, when compared to control and sham-lesioned rats, showed an inability to form and use a learning set. This research provided evidence that the nBM is critical for the formation of a learning set in rats. Support for the role

of cholinergic neurons in the nBM came from a study that tested the ability of nicotine, a nicotinic acetylcholine receptor agonist, to reverse the cognitive deficits associated with nBM lesions. The administration of nicotine to nBM-lesioned rats resulted in improved learning set performance (Lee et al., 2003).

Additional studies with 192-IgG saporin lesions to the nBM demonstrated that cholinergic neurons are important in the formation of a learning set due to the lesioned animals requiring more trials to acquire the task, but also indicated that cholinergic neurons are not the sole players, since the animals eventually formed and used a learning set despite the IgG saporin lesions (Bailey et al., 2003). These results suggest that another neurochemical system may be involved in the acquisition of learning set in rats. Martin et al. (2003) tested the possible involvement of the GABAergic system, and found improvement in learning set performance in nBM-lesioned animals following injections of flumazenil, a GABA antagonist. These results suggest a role for the GABAergic system in learning set performance.

Gamma-aminobutyric Acid (GABA)

GABA is an inhibitory neurotransmitter that is found in various regions throughout the brain, including the cerebral cortex, hippocampus, thalamus, basal ganglia, cerebellum, hypothalamus, and brainstem (Brambilla, Perez, Barale, Schettini, & Soares, 2003). Furthermore, the nucleus basalis magnocellularis (nBM), part of the basal forebrain system, contains GABAergic neurons, which outnumber acetylcholine neurons 2:1 (Gritti et al., 1993). GABA transmission is also evident in interneurons which modulate local neuronal circuitry, such as noradrenergic, dopaminergic, and serotonergic neurons (Brambilla et al., 2003).

GABA is synthesized from glutamate in an enzymatic reaction mediated by glutamic acid decarboxylase (GAD) (Brambilla et al., 2003). GABA can act on two different receptor types,

GABA_A and GABA_B receptors. The GABA_A receptors are ionotropic and are mostly located post-synaptically, causing fast inhibitory postsynaptic potentials (IPSP) (Eder, Rammes, Zieglansberger, & Dodt, 2001; as cited in Brambilla et al., 2003). Once the GABA_A receptor is activated either by GABA or by an agonist, such as muscimol, hyperpolarization of the cell can occur as a result of chloride channel opening (Brambilla et al., 2003).

Studies have found that the activity of cholinergic projecting neurons is regulated by cortical glutamatergic afferent systems and inhibitory GABAergic systems (Majchrzak et al., 1990). Wood (1985) evaluated the presence of different receptor populations in specific brain areas by locally injecting the appropriate receptor agonists and antagonists. Using this approach, Wood (1985) discovered that the nucleus basalis-cortical cholinergic pathway consists of an inhibitory GABAergic input to the nucleus basalis from the nucleus accumbens as well as positive glutamatergic feedback from the cortex. Furthermore, the septal-hippocampal cholinergic pathway also contains an inhibitory GABAergic regulatory system, consisting mainly of a large GABAergic interneuron population in the septum, with glutamatergic feedback from the hippocampus. Further studies indicated that injections of muscimol directly into the nBM resulted in a decrease in acetylcholine output (Casamenti, Deffenu, Abbamondi, & Pepeu, 1986). Casamenti et al. (1986) concluded that the GABA receptors in the nucleus basalis have the ability to inhibit the cholinergic neurons ascending to the cortex.

Muscimol

Muscimol is a GABA receptor agonist; specifically it works on the GABA_A subunit (Vazquez & Baghdoyan, 2004). Muscimol has been associated with aspects of learning, as well as being involved in the modulation of other neurotransmitters and the regulation of important life processes such as sleep. Vazquez and Baghdoyan (2004) tested the effect of bicuculline, a

GABA_A receptor antagonist, and muscimol on acetylcholine release in the cat pontine reticular formation, and used bicuculline doses of 0.03, 0.1, 0.3, 1, 3, and 10mM. Bicuculline resulted in a concentration dependent increase in acetylcholine release, and co-administration of bicuculline with muscimol blocked the bicuculline-induced increase in acetylcholine release. Furthermore, the 1 and 3 mM doses of bicuculline significantly increased acetylcholine release during wakefulness and during REM sleep, while also completely suppressing non-REM sleep. These results suggest that the GABA_A receptors have the ability to control REM sleep by inhibiting acetylcholine release in the reticular formation (Vazquez & Baghdoyan, 2004).

Injections of 1.0 and 2.0 mg/kg of muscimol have previously been shown to impair aspects of learning and memory including the retention of inhibitory avoidance (Morón et al., 2002), latent inhibition (Holt & Maren, 1999), retention of reward memory (Salinas & McGaugh, 1995), and place learning in the water maze (Nakagawa et al., 1995). Morón et al. (2002) tested the ability of male Wistar rats to learn an inhibitory avoidance task after intra-nBM injections of muscimol. Morón et al. (2002) tested six groups: a non-operated group, a cannulated non-injected group, a cannulated group receiving the vehicle (PBS; 0.8 µL per hemisphere), a cannulated group receiving bicuculline (0.05 µg in 0.8 µL per hemisphere), a cannulated group receiving muscimol (0.05 µg in 0.8 µL per hemisphere), and a cannulated group that received both bicuculline and muscimol (50% of each solution was mixed and administered in the above dose). The pharmacological agents were administered directly into the nBM prior to the acquisition of either of the learning tasks (conditioned taste aversion and inhibitory avoidance). The pharmacological agents were administered on day one 25 minutes prior to the delivery of the sodium saccharin solution for conditioned taste aversion, and 25 minutes prior to the training session on the conditioning day for the inhibitory avoidance

learning. Both bicuculline and muscimol microinjections resulted in no deficits in taste aversion learning, indicating that the GABAergic system of the nBM does not play a role in the acquisition of conditioned taste aversion learning. Furthermore, neither bicuculline nor muscimol resulted in any deficits of the acquisition of inhibitory avoidance learning. The researchers found that the rats receiving muscimol did not show any impairment in the acquisition of the task, but when the rats were tested 24 hours later, impairments in the inhibitory avoidance task were evident. Specifically, rats receiving muscimol spent less time in the safe compartment and had more crossings between compartments when compared to the non-operated group, the cannulated non-injected group, the group receiving the vehicle, and the group receiving both bicuculline and muscimol. These results suggest that muscimol may interfere with the consolidation processes necessary for inhibitory avoidance learning and does not interfere with the acquisition of inhibitory avoidance learning (Morón et al., 2002).

Holt and Maren (1999) tested the performance of rats on a latent inhibition task with either muscimol or saline infusions into the dorsal hippocampus. In the first experiment, one group of rats received a pre-exposure tone in the context that would later be used for extinction testing, a second group received a pre-exposure tone in a different context, and a third (control) group of rats did not receive the tone pre-exposure at all. All of the rats were then exposed to fear conditioning, consisting of tone-footshock pairs, in a third context distinct from either of the previous two contexts. On the next day, the researchers assessed conditional fear to the tone in one of the pre-exposure contexts by measuring extent of freezing during a tone extinction test. The researchers found that the rats, which were pre-exposed and tested in the same context, exhibited less freezing to the tone than the other two groups. Next, the researchers inactivated the dorsal hippocampus with muscimol (0.5 μ L of a 1 μ g/ μ L solution) 45 to 90 minutes prior to

the tone extinction test. The researchers used a range of times in order to determine a specific time course for the drug. The researchers found that this eliminated the context-specific expression of latent inhibition found in the first experiment. The rats that received muscimol exhibited low levels of tone freezing independent of whether they had received tone pre-exposure in the test context or in a different context. This study showed that muscimol had the ability to interfere with retention of memory for a latent inhibition task, and that the dorsal hippocampus is responsible for contextual retrieval for this task.

Salinas and McGaugh (1995) tested the effects of muscimol (1.0 and 3.0 mg/kg; i.p.) on memory for changes in reward magnitude. The researchers trained rats to run a straight alley for a large or a small food reward until the rats reached asymptotic performance. Once this occurred, the researchers switched the rats receiving the large food reward, so that they received a small food reward instead. Half of the animals received either 1.0 or 3.0 mg/kg (i.p.) of muscimol and the other half received comparable amounts of saline immediately after the training session. The researchers then continued training the rats on the shifted food reward for an additional three days without any injections. The researchers found that the rats that received 1.0 mg/kg of muscimol performed similar to saline controls, but those that received 3.0 mg/kg of muscimol showed significantly longer response latencies when tested on the second day after the shift in food reward. The researchers concluded that the post-training systemic administration of muscimol induces retrograde amnesia for food reward reduction.

Salinas and McGaugh (1995) also tested the ability of muscimol to affect the memory for food reward increase by first training the rats as previously noted. The researchers shifted the rats under the high reward condition to the small food reward. On the next training session, these rats were switched back to the large food reward condition, and were given an injection of

either 3.0 mg/kg (i.p.) of muscimol or comparable amounts of saline directly following this training session. All animals remained in the large food reward condition for the remainder of the testing sessions and received no further injections, as in the previous experiment. Compared to saline controls, the animals treated with muscimol again showed higher response latencies, indicating that muscimol could also induce retrograde amnesia for food reward increases. These researchers concluded that 3.0 mg/kg of muscimol is sufficient to interfere with memory of changes in food reward magnitude. However, these results must be analyzed with caution due to the lack of a measure of motility, since previous studies have shown that higher doses of muscimol can interfere with motor movement. Therefore, it is possible that the increased response latencies were observed due to the higher dose of muscimol impairing the motor movement, emotionality, or motivation of the animals.

Past studies have shown that the lateral and basal nuclei of the amygdala may be responsible for fear conditioning. Muller et al. (1997) tested the ability of muscimol to inactivate these nuclei, and whether or not this resulted in a deficit in fear conditioning. Male Sprague-Dawley rats were either infused with muscimol or saline just prior to fear conditioning, and then they received another infusion approximately 24 hours later when freezing in response to the tone conditional stimulus (CS) was tested in a novel context. The rats either received 0.5 μ L of 0.9% saline vehicle or the vehicle plus 0.5 μ g of muscimol; both agents were injected bilaterally through infusion cannulae into the amygdala. The research consisted of four groups: 1) saline before training and before testing (saline-saline), 2) saline-muscimol, 3) muscimol-saline, and 4) muscimol-muscimol. The muscimol-muscimol group was a control for state-dependent learning, because close inspection of this group would reveal if performance was differentially affected by the time at which muscimol was delivered. In addition, by comparing the saline-muscimol and

muscimol-saline groups, one could see the effects on both acquisition and expression of conditioned fear.

Muller et al. (1997) found that the rats that received muscimol injections either prior to training or testing showed significantly reduced levels of freezing to the tone and to the context alone. The researchers retested the rats, which had originally received muscimol, with saline to test whether the effects observed with muscimol were reversible. The researchers showed that the effects of muscimol indeed were not permanent due to the rats' tendency to perform at levels not significantly different from saline-saline controls when retested with saline. These results indicated that the impairments produced by muscimol were reversible and the inactivation of the lateral and basal nuclei of the amygdala by muscimol before training disrupted fear learning in the rats. The saline-muscimol group apparently had learned the fear conditioned response but could not express it when tested with muscimol, as evidenced by the proper expression of the fear conditioned response when later retested with saline infusions. This study provided evidence for the ability of muscimol to block both learning and expression of a fear-conditioning task in rats (Muller et al., 1997).

Nakagawa et al. (1995) investigated the effects of muscimol on place learning in the Morris water maze in rats in order to determine whether muscimol produced effects by inducing state-dependent learning. The rats were given four training trials per day with the platform submerged at a fixed location. On the fourth day, the rats were required to swim without the platform after four trials, as a probe test. Rats which were treated with muscimol on days 1-4 did not appear to be impaired in the water maze task, compared to saline-treated controls. However, when rats were treated with muscimol on days 1-3 and saline on day 4, impairments were observed on day 4, as evidenced by the rats requiring a longer time to reach the platform and

spending less time in the quadrant where the platform was located. When bicuculline was administered with muscimol on days 1-3, and saline was injected on day 4, the deficits produced by muscimol alone were reversed. Specifically, these rats did not require a longer time to reach the platform and spent approximately the same amount of time in the quadrant where the platform was located as saline controls. Furthermore, when muscimol was administered on days 1-3, and bicuculline was co-administered with muscimol on day 4, place learning was blocked. The researchers concluded that muscimol acts by inducing state-dependent learning in the Morris water maze, due to its differential effects depending on the time of administration and the agents with which it is co-administered.

Majchrzak et al. (1990) investigated the effects of GABA on sensorimotor and cognitive skills of male Long-Evans rats, using the radial arm maze and a passive avoidance task. The doses of GABA used were $10\mu\text{g}/\mu\text{L}/\text{h}$ and $50\mu\text{g}/\mu\text{L}/\text{h}$, and the GABA was infused into the nBM using cannulae and osmotic minipumps. Rats were allowed to train to criterion in the radial arm maze task and the rats were then infused with GABA ($10\mu\text{g}/\mu\text{L}$ or $50\mu\text{g}/\mu\text{L}$) or saline one day after they reached criterion. The GABA was administered every day of the radial arm maze testing. During the radial arm maze task, GABA-treated rats made significantly more errors than the saline controls, indicating cognitive impairments. However, only the rats with the highest doses of GABA took more time to run the maze, indicating a performance problem with the higher dose. For the passive avoidance task, the administration of GABA during the acquisition phase resulted in GABA-treated rats requiring more trials to reach criterion levels. All groups eventually learned the task, but it took significantly longer for the GABA-treated rats to learn. Majchrzak et al. (1990) also found no effect of GABA infusions on the retention of the passive avoidance task.

Majchrzak et al. (1990) also tested the effect of GABA infusions on the retention of the passive avoidance task by testing the retention 48 hours after acquisition to criterion. There appeared to be no effect of GABA infusions on the retention of the passive avoidance task once it was learned. However, the deficit in the acquisition of the passive avoidance task continued for one week after treatment ended, suggesting a relatively long lasting effect of GABA. In addition, there appeared to be no effect of GABA on the ability of the rats to relearn the task to criterion after it had been extinguished.

Muscimol has also been implicated in retrograde amnesia when infused into the hippocampus. Rossato et al. (2004) infused muscimol into the dorsal CA1 region of the hippocampus, as well as into the basolateral amygdala, and the entorhinal, parietal, and posterior cingulate cortices of male Wistar rats, and tested the animals on a 1-trial inhibitory avoidance task. The rats were trained in a step-down inhibitory avoidance task three to five days following the implantation of the cannulae. Either immediately or 30, 90, 180, 270, or 360 minutes following training, the animals were administered a bilateral 0.5 μ L infusion of vehicle, AP5 (specific antagonist for glutamate NMDA receptors; 2.5 μ g per side), muscimol (0.3 μ g per side) or UO 126 (specific inhibitor of MEK1/2, upstream regulators of the mitogen-activated protein kinases-ERK1/2 pathway; 0.4ng per side) dissolved in vehicle. The researchers found that muscimol induced amnesia when tested on this one-trial inhibitory avoidance task, and concluded that GABA_A receptor activation may inhibit the memory consolidation process, especially since the muscimol appeared to have the most profound negative effects when administered immediately after training.

Cooke et al. (2004) also tested the ability of muscimol to interfere with cerebellar-dependent memory consolidation in rabbits. The researchers infused muscimol (3mM; 2 μ L in

0.01 M PBS) or saline (2 μ L) directly into the right cerebellar cortical lobule HVI at 5, 45, or 90 minutes after the end of the training session. All animals then received four more daily sessions of training with no post-training infusions and then the rabbits receiving muscimol received four additional daily sessions of training without infusions. The researchers also tested the effects of muscimol at 5 and 30 minutes, and at 1, 2, 4, 8, and 24 hours post-infusion. Cooke et al. (2004) discovered that cortical infusions delivered 5 or 45 minutes after a conditioning session produced significant impairments in memory consolidation, but infusions delivered 90 minutes after the session produced little or no impairment. Furthermore, the researchers discovered that muscimol infusions produced significant impairments after 30 minutes, and these effects lasted for a few hours, with a complete rebound in performance occurring after 24 hours.

Conclusion

In conclusion, the present study investigated the effect of muscimol on the acquisition of a learning set in male Long-Evans rats. Learning set was tested using 40 odor-unique discrimination problems (following Bailey & Thomas, 2001). During this acquisition phase, rats were randomly chosen to receive either muscimol (0.5 mg/kg; i.p.) or comparable amounts of saline. Once all animals had completed these 40 odor-unique discrimination problems, they received an additional 30 problems with no injections. Open field tests were performed during the pre-treatment, treatment, and post-treatment phases to examine possible motility and emotionality differences in muscimol- and saline-treated rats. Based on previous research findings indicating the ability of muscimol to interfere with acquisition in other learning paradigms, it was hypothesized that muscimol would significantly impair the ability of rats to acquire an olfactory discrimination learning set. However, 0.5 mg/kg injections of muscimol did not effect the acquisition of a learning set, activity levels, or emotionality.

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